Electrophysiological responses of canine atrial endocardium and epicardium to acetylcholine and 4-aminopyridine

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Abstract

Objectives: Prior studies demonstrated marked electrophysiological and pharmacological differences between canine ventricular epicardium and endocardium. For atrium, however, it has been assumed that, because of the thin wall, electrical properties of epicardium and endocardium are similar. The aim of the present study was to compare the action potential (AP) characteristics in epicardial and endocardial atrial cells before and following addition of acetylcholine (ACh) and 4-aminopyridine (4-AP).

Methods and Results: Microelectrode techniques were used to study the effects of ACh (10^{-7}–10^{-5} M) and 4-AP (0.5 mM) on epicardial and endocardial AP of canine right atrial free wall at cycle lengths (CL) of 250 to 2000 ms. ACh hyperpolarized epicardial and endocardial cells (by 5–8 mV at 10^{-5} M). In control, AP duration to 90% repolarization (APD_{90}) was longer in endocardium at all CL. ACh shortened APD in either tissue with more prominent effect in endocardium (at 10^{-5} M and CL=2000 ms, from 179±10 to 90±11 ms in epicardium and from 209±10 to 65±6 ms in endocardium, P<0.05). As a result, at 10^{-5} M, APD_{90} in endocardium was shorter than in epicardium at all CL. 4-AP effects on AP duration were similar in both tissue types. No effects of 4-AP was seen at CL=250 ms and at long CL, the compound shortened APD_{90} and prolonged AP duration to 50% repolarization. Conclusions: (1) ACh exerts direct effects on atrial epicardial and endocardial AP; (2) 4-AP-sensitive transient outward current (I_{to}) is expressed both in canine atrial epicardial and endocardial cells; (3) differential response of epicardial and endocardial APD to ACh may alter the gradient of repolarization across the atrial wall and contribute to vagally induced atrial flutter and fibrillation. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Canine atrium; Epicardium; Endocardium; Action potential; Acetylcholine; 4-Aminopyridine

1. Introduction

Marked differences between the action potential characteristics of canine ventricular endocardium and epicardium have been described [1]. A major mechanism responsible for the electrophysiological dissimilarities is the presence of a prominent 4-aminopyridine (4-AP)-sensitive transient outward current (I_{to}) in epicardium but not in endocardium [2]. However, differences in other ionic currents may contribute to these distinctions as well [1]. Due to the electrophysiological dissimilarities, epicardial and endocardial cells show different – in some cases opposite – responses to a variety of pharmacological agents including neurotransmitters [1,3]. For example, acetylcholine (ACh) has no consistent effect on the action potential of canine ventricular endocardium but, depending on concentration and pacing cycle length, either prolongs or shortens action potential duration (APD) of epicardium [3].

In the case of a thin-walled chamber like the atrium, it has been assumed that the wall can be considered a sheet and that no differences exist between cells of endocardial and epicardial surfaces. However, there is no experimental evidence for this assumption. Moreover, bilateral mapping...
The isolated canine right atrium during fibrillation induced in the presence of ACh has demonstrated that epicardial and endocardial activation can be discordant and that reentry can occur in the atrium as in a three-dimensional structure [4,5]. Although the differences in the epicardial and endocardial activation may result from the heterogeneity of the anatomic architecture of the atrium [5], a differential response of atrial epicardium and endocardium to ACh can also be hypothesized. Therefore, the present study was designed to investigate the effects of ACh on action potential configuration in isolated epicardial and endocardial canine atrial tissues. Because in the ventricle $I_{\text{Na}}$ is present in epicardium, but not in endocardium, effects of $I_{\text{Na}}$ blocker 4-AP were also compared in the epicardium and endocardium of the atrium.

2. Methods

All experimental procedures conformed to the Guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH publication No 85-23, revised 1985).

Mongrel dogs of either sex weighing 10–15 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Their hearts were removed through a left lateral thoracotomy and immersed in cold Tyrode’s solution equilibrated with 95% O$_2$–5% CO$_2$ and containing (mM): NaCl 131, NaHCO$_3$ 18, KCl 4, CaCl$_2$ 2.7, MgCl$_2$ 0.5, NaH$_2$PO$_4$ 1.8 and dextrose 5.5. Atrial strips (~10×10 mm) were dissected from the free wall of the right atrium, placed in a tissue bath epicardial or endocardial surface up and superfused with Tyrode’s solution warmed to 37°C (pH 7.35±0.05). Solution was pumped through the tissue bath at a flow-rate of 12 ml/min, changing chamber content three times per minute. The bath was connected to ground via a 3 M KCl/Ag/AgCl junction.

All preparations were impaled with 3 M KCl-filled glass capillary microelectrodes that had tip resistances of 10 to 20 MΩ. The maximum upstroke velocity of the action potential ($V_{\text{max}}$) was obtained by electronic differentiation with an operational amplifier. The electrodes were coupled by an Ag/AgCl junction to an amplifier with high input impedance and input capacity neutralization. Transmembrane action potentials and $V_{\text{max}}$ signals were digitized with an analog-to-digital converter (D-210, DATAQ Instruments) and stored to a computer for subsequent analysis. For stimulation of preparations, standard techniques were used to deliver square-wave pulses 1.0 ms in duration and 1.5 times threshold through bipolar PTFE-coated silver electrodes. To investigate frequency-dependence of drug effects, the preparations were driven at cycle lengths of 2000, 1000, 500 and 250 ms in sequence. Each frequency was maintained for 3 min before data were collected.

Experiments were started after preparations had fully recovered and displayed stable electrophysiological characteristics which required 2 h of superfusion in control Tyrode’s solution. After control records were obtained, the preparations were superfused with Tyrode’s solution containing graded concentrations ($10^{-7}$, $10^{-6}$ and $10^{-5}$ M) of ACh. Because preliminary experiments had shown that steady-state effects on action potential parameters were achieved in 3 to 5 min, the frequency scan was started after the preparations were equilibrated at each drug concentration for 5 min. After the highest concentration was examined, the preparations were superfused with control Tyrode’s solution for 5 min (washout) and action potential parameters were recorded. To study the effects of 4-AP (0.5 mM), the compound was added to Tyrode’s solution, and after 10 min the frequency scan was obtained.

Acetylcholine HCl (Sigma) was dissolved in distilled water to yield a stock solution of 1 mM. 4-Aminopyridine (Sigma) was dissolved in distilled water and made soluble by warming to yield a stock solution of 0.5 M. The pH of stock solution was adjusted to 7.4 with HCl.

Microelectrode data were analyzed from impalements maintained throughout the course of each experiment. Data are expressed as mean±S.E.M. The statistical techniques used were one-way or two-way analysis of variance for repeated or nonrepeated measures, with Bonferroni’s test when the $F$-value permitted [6]. Significance was determined at $P<0.05$. 

![Fig. 1. Representative experiments illustrating the effects of $10^{-5}$ M acetylcholine (ACh) on transmembrane action potential in epicardial and endocardial tissues driving at cycle lengths (CL) of 250 ms (left panels) and 2000 ms (right panels). In each panel, top trace shows transmembrane action potential and bottom trace shows $V_{\text{max}}$. C, control. Vertical calibration is for action potential and $V_{\text{max}}$; horizontal for action potential.](image-url)
Table 1
Effects of acetylcholine on selected action potential parameters of atrial epicardium

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>10^{-3} M</th>
<th>10^{-5} M</th>
<th>10^{-7} M</th>
<th>Washout</th>
</tr>
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<tbody>
<tr>
<td>CL 250 ms</td>
<td>MDP (mV)</td>
<td>75±1</td>
<td>75±2</td>
<td>79±2</td>
<td>82±1</td>
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<td></td>
<td>APA (mV)</td>
<td>87±3</td>
<td>88±3</td>
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<td>V_{max} (V/s)</td>
<td>174±18</td>
<td>176±16</td>
<td>209±19</td>
<td>233±20*</td>
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<td>CL 500 ms</td>
<td>MDP (mV)</td>
<td>76±1</td>
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<td>80±2*</td>
<td>83±2*</td>
</tr>
<tr>
<td></td>
<td>APA (mV)</td>
<td>89±3</td>
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<td>96±2*</td>
<td>96±2*</td>
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<tr>
<td></td>
<td>V_{max} (V/s)</td>
<td>194±15</td>
<td>203±11</td>
<td>230±22</td>
<td>244±21*</td>
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<tr>
<td>CL 1000 ms</td>
<td>MDP (mV)</td>
<td>76±1</td>
<td>77±2</td>
<td>82±2*</td>
<td>83±2*</td>
</tr>
<tr>
<td></td>
<td>APA (mV)</td>
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<td>98±2*</td>
</tr>
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<td>V_{max} (V/s)</td>
<td>192±17</td>
<td>201±17</td>
<td>235±20</td>
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<tr>
<td>CL 2000 ms</td>
<td>MDP (mV)</td>
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<td>76±2</td>
<td>83±2*</td>
<td>84±2*</td>
</tr>
<tr>
<td></td>
<td>APA (mV)</td>
<td>89±3</td>
<td>92±3</td>
<td>98±3*</td>
<td>99±2*</td>
</tr>
<tr>
<td></td>
<td>V_{max} (V/s)</td>
<td>186±16</td>
<td>218±16</td>
<td>248±20*</td>
<td>256±27*</td>
</tr>
</tbody>
</table>

MDP = maximum diastolic potential; APA = action potential amplitude; V_{max} = maximum rate of rise of phase zero; CL = cycle length of stimulation.

Values are mean±S.E.M. (n=10).

*P<0.05 vs. control at the same CL (one-way ANOVA for repeated measures).

3. Results

Fig. 1 illustrates representative transmembrane potentials recorded in control and in the presence of Ach, 10^{-5} M, at the longest and shortest CLs from epicardial and endocardial cells. Data summarizing the effects of varying concentrations of ACh on maximum diastolic potential, action potential amplitude and V_{max} in all experiments at all CLs are shown in Tables 1 and 2. The effects of ACh on all these parameters were quantitatively similar in epicardial and endocardial cells: the compound hyperpolarized atrial cells in a concentration-dependent manner and

Table 2
Effects of acetylcholine on selected action potential parameters of atrial endocardium

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>10^{-3} M</th>
<th>10^{-5} M</th>
<th>10^{-7} M</th>
<th>Washout</th>
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<tr>
<td>CL 250 ms</td>
<td>MDP (mV)</td>
<td>77±1</td>
<td>78±1</td>
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<td></td>
<td>APA (mV)</td>
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<td>168±14</td>
<td>179±15</td>
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<tr>
<td>CL 500 ms</td>
<td>MDP (mV)</td>
<td>79±1</td>
<td>81±1</td>
<td>83±1*</td>
<td>84±1*</td>
</tr>
<tr>
<td></td>
<td>APA (mV)</td>
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<td>96±2</td>
<td>98±2</td>
<td>99±2*</td>
</tr>
<tr>
<td></td>
<td>V_{max} (V/s)</td>
<td>190±17</td>
<td>231±15</td>
<td>256±15*</td>
<td>247±12*</td>
</tr>
<tr>
<td>CL 1000 ms</td>
<td>MDP (mV)</td>
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<td>80±1</td>
<td>84±1*</td>
<td>85±1*</td>
</tr>
<tr>
<td></td>
<td>APA (mV)</td>
<td>95±3</td>
<td>97±2</td>
<td>100±2</td>
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</tr>
<tr>
<td></td>
<td>V_{max} (V/s)</td>
<td>203±15</td>
<td>210±20</td>
<td>227±20</td>
<td>238±14*</td>
</tr>
<tr>
<td>CL 2000 ms</td>
<td>MDP (mV)</td>
<td>77±1</td>
<td>79±1</td>
<td>84±1*</td>
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<tr>
<td></td>
<td>APA (mV)</td>
<td>94±3</td>
<td>97±3</td>
<td>103±2*</td>
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<td></td>
<td>V_{max} (V/s)</td>
<td>201±17</td>
<td>230±17</td>
<td>235±17</td>
<td>250±18*</td>
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</tbody>
</table>

MDP = maximum diastolic potential; APA = action potential amplitude; V_{max} = maximum rate of rise of phase zero; CL = cycle length of stimulation.

Values are mean±S.E.M. (n=11).

*P<0.05 vs. control at the same CL (one-way ANOVA for repeated measures).
induced an increase of action potential amplitude and $V_{\text{max}}$. These effects did not depend on stimulus rate. After a 5-min washout period (superfusion with control Tyrode’s solution) AP parameters were close to control values.

The most prominent effect of ACh was a marked abbreviation of the APD in either tissue. The shortening was observed at both 50 and 90% of full repolarization (Fig. 1), was concentration-dependent (Fig. 2) and rate independent (Fig. 3). Qualitatively the effects were similar in both types of tissues. However, quantitative differences were clearly seen: the effects of ACh to decrease the APD were more prominent in endocardium in comparison to epicardium. In control, action potential duration to 90% repolarization ($\text{APD}_{90}$) was significantly longer in endocardium at all cycle lengths (Fig. 3A). In the presence of ACh, $10^{-5}$ M, the opposite relationship was observed: the $\text{APD}_{90}$ in endocardial cells was significantly shorter than in epicardial cells. Although not reaching statistical significance, the same relationship between endocardial and epicardial APD was observed at the level of 50% repolarization ($\text{APD}_{50}$) (Fig. 3B). The effects of ACh were completely reversed after the addition of the muscarinic blocker atropine, $10^{-6}$ M.

In another series of experiments, the effects of $I_{\text{to1}}$ blocking agent 4-AP (0.5 mM) were examined. Representative experiments with epicardial and endocardial tissues are pictured in Fig. 4. The compound slowed the early repolarization and accelerated the rate of final repolarization in either tissue. The actions of 4-AP began in the first few minutes and stabilized after 7 to 10 min. They were reversible within 15 to 20 min. The effects of 4-AP on repolarization were rate-dependent and increased with slowing stimulation frequency (Fig. 4). As a result, the compound had no effects on APD at the shortest cycle length and significantly prolonged the $\text{APD}_{50}$ and reduced the APD at voltages near the resting potential ($\text{APD}_{90}$) at long cycle lengths (Fig. 5). Quantitatively 4-AP effects on repolarization were similar in epicardial and endocardial cells. 4-AP had no effects on maximum diastolic potential and $V_{\text{max}}$ in either tissue at any cycle length (data are not

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**Fig. 2.** Concentration-dependent effects of acetylcholine (ACh) on action potential duration to 90% repolarization ($\text{APD}_{90}$) in epicardial (epi) and endocardial (endo) tissues at cycle lengths (CL) of 250 ms (A) and 2000 ms (B). Values are mean±S.E.M. ($n=10$ for epi and $n=11$ for endo). *$P<0.05$ vs. respective control (one-way ANOVA for repeated measures). **$P<0.05$ vs. epi at the same ACh concentration (two-way ANOVA for nonrepeated measures).
shown) and induced a small but statistically significant increase of action potential amplitude at the longest cycle length (from 90±4 to 97±3 mV in epicardium, and from 93±2 to 99±3 mV in endocardium, $P<0.05$ for both).

4. Discussion

To our knowledge the transmembrane potential from atrial epicardial cells has never been recorded and, therefore, the comparison of action potentials of atrial epicardium and endocardium has not been previously reported. The results of the present study are generally consistent with the assumption that, because of the thin wall, the electrophysiological properties of epicardial and endocardial cells of the atrium have to be similar. However, our findings clearly demonstrate that these properties are not identical. We have found that under control conditions there is a difference between durations of action potentials recorded from epicardial and endocardial surfaces of the same region of the right atrial free wall: endocardial APD is longer than epicardial at all cycle lengths.

The effects of ACh on maximum diastolic potential, action potential amplitude and $V_{\text{max}}$ are similar in atrial epicardium and endocardium and differ from its effects in the ventricles. In contrast to the ventricles, where ACh has no effects on maximum diastolic potential in both epicardium and endocardium [3], it significantly hyperpolarizes atrial tissues. This result is in a good agreement with the data by Glitch and Pott [7] who observed 6–7 mV hyperpolarization in isolated guinea pig right atrium in response to ACh ($10^{-6}$ and $10^{-5}$ M) or stimulation of parasympathetic nerve fibers. The discordance of ACh effects on resting potential in atrium and ventricle can be explained by the differences in potassium conductances between these tissues. It has been shown that the inward rectifier potassium current ($I_{K1}$) is more prominent in ventricular than in atrial myocytes [8,9] whereas sensitivity to ACh and density of ACh-activated potassium channels ($I_{K(ACh)}$) are greater in atrial tissue [10]. Thus, $I_{K1}$ channels are responsible for the basal potassium conductance in ventricle, whereas in atrium, although $I_{K1}$ channels contribute significantly to basal potassium current [11], the relative amplitude of the $I_{K1}$ and $I_{K(ACh)}$ are of comparable size [11,12] and resting membrane potential can be significantly modulated by ACh [11–13]. The increases of $V_{\text{max}}$ and action potential amplitude at high ACh concentrations are, most likely, a result of hyperpolarization of the membrane induced by the compound.

The most interesting findings of the present study are the effects of ACh on APD in atrial epicardium and endo-
cardium. We have shown that, in contrast to the ventricles, where ACh affects APD in epicardium, but not in endocardium [3], it concentration-dependently abbreviates APD in both atrial tissues. The available data suggest that ACh-induced APD shortening in atrial tissue is largely due to activation of \( I_{K(ACh)} \) [11–13] and, probably, inhibition of slow inward calcium current \( (I_{Ca}) \) [14,15]. The ability of ACh to shorten APD is more prominent in endocardium than in epicardium. As a result, ACh can significantly change and even reverse a gradient of repolarization across atrial wall: endocardial APD is longer than epicardial at all cycle lengths in the absence of ACh and opposite relationship is seen at high \( (10^{-5}) \) ACh concentration. The precise mechanisms responsible for the difference of sensitivities between epicardial and endocardial APD to ACh are not clear; however, some speculations are in order. First, the density of muscarinic receptors and/or ACh-affected ionic channels can be different in epicardial and endocardial regions. Second, the differences in the ionic basis of the action potentials in epicardium and endocardium under control conditions can influence their responses to ACh. Finally, it has been shown that substances which can be released by endocardial endothelium in response to muscarinic stimulation (such as endothelin and nitric oxide) activate \( I_{K(ACh)} \) and inhibit \( I_{Ca} \) in mammalian atrial myocytes [16,17] and can, therefore, accentuate ACh effect on APD in endocardium. These hypotheses need testing.

The present study shows that, in contrast to the ventricles where 4-AP has prominent effect in epicardium, but not in endocardium [2], its action in atrial epicardium and endocardium is similar. This result suggests that 4-AP-sensitive transient outward current \( (I_{to}) \) is almost identically expressed in both types of atrial cells. The effects of 4-AP on atrial repolarization can be explained as follows. The blocker inhibits the fast and large component of transient outward current [18–20] and broadens the action potential at plateau level. The APD widening at plateau level speeds activation of delayed rectifier potassium current \( (I_K) \), which has been identified in atrial cells [19–21], and accelerates the final phase of repolarization. As a result, APD_{50} prolongation and APD_{90} shortening are observed. Similar changes of the action potential configuration in the presence of 4-AP was shown by Shibata et al. [19] in human atrial endocardium. Because \( I_{to} \) slowly recovers from inactivation and decreases with an increase of stimulation rate [19], the effects of 4-AP on repolarization are prominent at long cycle lengths.

The physiological implications of the present findings relate to the mechanisms of atrial arrhythmias. It has been shown that cholinergic influences (vagal stimulation or application of ACh) induce supraventricular tachyarhythmias including atrial flutter and fibrillation in isolated atrium of frog [22], rabbit [23], and dog [4,24] and in the dog heart in situ [25]. The most possible mechanism is reentry and spatial heterogeneity in atrial repolarization [26,27] as well as non-uniform distribution of vagal innervation and its effects on repolarization over the atrial muscle [28,29] have been suggested to play an important role in initiation and maintaining of the reentrant arrhythmias. We speculate that the differential influence of ACh on repolarization in epicardium and endocardium observed in the present study may contribute to asymmetry between epicardial and endocardial activations and promote reentry in the atrium as in a three-dimensional structure [4,5]. On the other hand, there are indications that parasympathetic ganglia are predominantly located within the subepicardial connective tissue of the atria [29] which can suggest a nonuniform distribution of postganglionic terminal fibers within the atrial wall. More abundant innervation of epicardium can compensate its lower sensitivity to ACh and equalize vagal effects on repolarization across the atrial wall in situ. Further work is necessary to evaluate this speculation.

Acknowledgements

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References


